THE RELATION BETWEEN HYDROGEN-ION CONCENTRATION AND VOLUME, GEL/SOL RATIO AND ACTION OF THE CONTRACTILE VACUOLE IN AMOEBA PROTEUS [1]

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Introduction

Von Limbeck (1894) observed that erythrocytes swell if the concentration of carbon dioxide in the blood is increased. Jacobs and Parpart (1931) found that hemolysis in erythrocytes increases with increase in acidity and that the effect of as small a change as 0.01 pH is measurable. Lucké and McCutcheon (1926) maintain that the volume of eggs of Arbacia in sea water does not change with changes in hydrogen-ion concentration between pH 4.2 and pH 9.8 unless the eggs are left so long that they become injured.

Chalkley (1929), in observations on *Amoeba proteus* in balanced salt solution, found that as the hydrogen-ion concentration decreases from pH 6 the volume decreases to a minimum at pH 7 and then increases, i.e. that there are two maxima, one in the acid range and another in the alkaline; and Mast and Prosser (1932) found that as it decreases over the range studied (pH 5.4–8) the gel/sol ratio decreases. Pitts and Mast (1934) investigated the relation between gel/sol ratio and hydrogen-ion concentration in single as well as balanced salt solutions. They confirmed the results obtained by Mast and Prosser and conclude that " in sodium or potassium salt solutions the gel/sol ratio decreases as the hydrogen-ion concentration decreases, but that in calcium salt solutions it increases in the more acid range (pH 5.0 to pH 5.9) then remains constant or decreases slightly."

Thus it will be seen that there is considerable difference of opinion concerning the effect of changes in hydrogen-ion concentration of the medium on the volume of cells, that no observations have been made on *Amoeba* concerning the relation between volume and hydrogen-ion con-

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centration in single salt solutions, and that the results obtained in observations on the gel/sol ratio in these solutions have not been confirmed. It is the purpose of this paper to present detailed information concerning the relation between hydrogen-ion concentration, volume and the gel/sol ratio of *Amoeba proteus* in salt solutions containing respectively sodium, potassium and calcium as the only metallic cations.

Material and Methods

The amoebae used were selected, prepared, and measured for volume and gel/sol ratio as described in the section on material and methods in a previous paper (Mast and Fowler, 1935). The solutions used consisted of a primary phosphate hydroxide buffer system in which the concentration of the cation was identical in the phosphate and in the hydroxide (Pitts and Mast, 1933). The stock solution of phosphate was kept in a covered Pyrex flask and the stock solution of hydroxide in a carefully sealed Pyrex flask open to the exterior through a soda lime tube and through a 50 cc. Pyrex glass buret. These solutions were standardized according to the method described by Pitts and Mast (1933). By mixing the phosphate and the hydroxide in various proportions the desired hydrogen-ion concentration was easily obtained. The hydrogen-ion concentration of each solution prepared was measured with a quinhydrone electrode and a Leeds Northrup potentiometer.

Volume and Gel/Sol Ratio

Sodium Salts

Ten amoebae were selected, put into modified Ringer solutions,[2] and left for approximately 24 hours. Then the volume and the gel/sol ratio of each were measured as described above, after which they were transferred to a solution containing 0.002 M sodium as the only metallic cation at pH 5.5, left 15 minutes and measured again, after which they were measured at 15-minute intervals for 105 minutes. This was repeated for 60 other individuals, 10 in each of the following solutions: 0.002 M sodium phosphate buffer solutions at pH 6.0, pH 6.5, pH 7, pH 7.5, pH 8.0 and pH 8.8, respectively. There was but little change in either the volume or the gel/sol ratio of the amoebae after they had been in these solutions 30 minutes. All the results obtained in the

[2] 3.3 cc. salt solution (0.35 gram NaCl, 0.14 gram KCl, 0.12 gram $CaCl_2$, 1000 cc. H_2O) + 5 cc. buffer solution (25 cc. 0.2 M KH_2PO_4, 12.5 cc. 0.2 M NaOH, 62.5 cc. H_2O; Clark, 1927) + 91.7 cc. H_2O. This solution is the same in composition as Chalkley's 1/60 Ringer solution (1929). The total concentration of salts is 0.002 M and the hydrogen-ion concentration is pH 6.8.

measurements of volume and gel/sol ratio made at each hydrogen-ion concentration were therefore respectively thrown together and the average calculated. These averages are presented in Fig. 1, *A*.

Figure 1, *A* shows that after the amoebae had been transferred from modified Ringer solution 0.002 M, pH 6.8, to 0.002 M sodium phos-

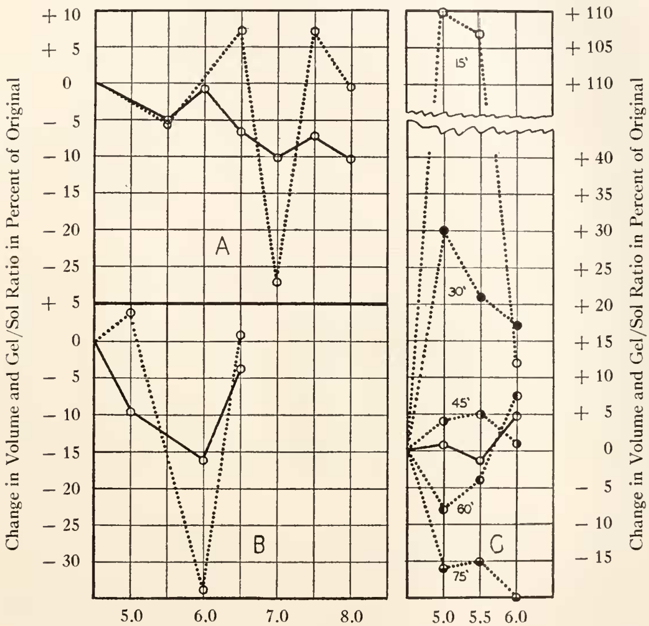

FIG. 1. The relation between volume and gel/sol ratio in *Amoeba proteus* and hydrogen-ion concentration in solutions which contain only one kind of metallic cations. *A*, sodium 0.002 M; *B*, potassium 0.002 M; *C*, calcium 0.005 M; solid curves, volume; broken curves, gel/sol ratio; 0, volume and gel/sol ratio in modified Ringer solution; +, increase in volume and gel/sol ratio; —, decrease in volume and gel/sol ratio; points on curves, averages of 3 to 50 measurements (see text); 15′, 15 min. after transfer from Ringer solution to Ca solution; 30′, 30 min.; 45′, 45 min.; 60′, 60 min.; 75′, 75 min.

phate buffer solutions at the various hydrogen-ion concentrations used, the average volume calculated from measurements made at 15-minute intervals for 105 minutes after transfer, decreased 5 per cent at pH 5.5, 0.3 per cent at pH 6.0, 7 per cent at pH 6.5, 10 per cent at pH 7.0, 7.3 per cent at pH 7.5 and 10 per cent at pH 8.0. (Measurements at pH 8.8 were impossible because the amoebae disintegrated within a few minutes after they had been put into the solutions.)

This indicates that as the hydrogen-ion concentration in a sodium solution decreases from pH 5.5 the volume increases to a maximum at pH 6, then decreases to a minimum at pH 7, then increases to a secondary maximum at pH 7.5, and then decreases again.

Figure 1, *A* also shows that the gel/sol ratio decreased 5.5 per cent at pH 5.5, 27 per cent at pH 7 and zero per cent at pH 8, and that it increased 7.5 per cent at pH 6.5 and pH 7.5. This indicates that in a sodium solution as the hydrogen-ion concentration decreases from pH 5.5 the gel/sol ratio increases to a maximum at pH 6.5, then decreases rapidly and very greatly to a minimum at pH 7, then increases equally rapidly and greatly to a second maximum at pH 7.5 and then decreases again.

Potassium Salts

The experiments concerning the relation between volume and gel/sol ratio and hydrogen-ion concentration in solutions containing potassium as the only metallic cation were performed the same as those containing only sodium, except that three specimens were used for each hydrogen-ion concentration in place of ten. The results obtained show that there was, as in the sodium solutions, but little change in volume and gel/sol ratio after the amoebae had been in the solutions 15 minutes. The averages of all the results obtained concerning volume and gel/sol ratio were therefore respectively calculated. These averages are presented in Fig. 1, *B*.

Figure 1, *B* shows that in amoebae transferred from modified Ringer solution, pH 6.8, to potassium phosphate buffer solutions at various hydrogen-ion concentrations, the volume decreased 10 per cent at pH 5.0, 16.1 at pH 6.0, and 3.7 at pH 6.5, and that the gel/sol ratio increased 4 per cent at pH 5 and about 1 at pH 6.5 and decreased 33.8 per cent at pH 6. In neutral and alkaline solutions the amoebae disintegrated so rapidly that it was impossible to measure them.

These results indicate that as the hydrogen-ion concentration in potassium solutions decreases from pH 5 the volume of *Amoeba proteus* decreases slowly to a minimum at pH 6 and then increases rapidly; and that the gel/sol ratio decreases very rapidly to a minimum at pH 6 and then increases equally rapidly.

Calcium Salts

The methods used in the observations on amoebae in solutions in which calcium was the only metallic cation present are the same as those used in the preceding experiments. The concentration of calcium was 0.005 M and ten amoebae were measured at each of four hydrogen-ion concentrations: pH 5, 5.5, and 6. In lower hydrogen-ion concentrations, it was impossible to maintain the concentrations long enough to make the measurements.

The results obtained show that there was but little change in volume after the amoebae had been in the solutions 15 minutes, but that the gel/sol ratio changes radically with time. In reference to volume, the average for all the measurements made at each hydrogen-ion concentration was therefore calculated; but in reference to gel/sol ratio the average of the results obtained in the measurements made after each 15-minute period at each hydrogen-ion concentration was calculated. These averages are presented in Fig. 1, *C*.

Figure 1, *C* shows that in the amoebae which were transferred from modified Ringer solution pH 6.8 to calcium phosphate buffer solutions the volume increased 0.8 per cent at pH 5 and 4.8 per cent at pH 6 and that it decreased 1.4 per cent at pH 5.5. This indicates that as the hydrogen-ion in calcium solutions decreases from pH 5 the volume decreases slightly to a minimum at pH 5.5 and then increases fairly rapidly.

This figure shows that during the first 15 minutes after the amoebae had been transferred from modified Ringer solution the gel/sol ratio increased 110 per cent at pH 5.5, 107 per cent at pH 6.5 and 12 per cent at pH 6, and that it then decreased during the following 90 minutes to 16 per cent below the original ratio at pH 5, 15 per cent at pH 5.5 and 20 per cent at pH 6.

This indicates that after transfer from Ringer solution to calcium solution the gel/sol ratio increases very rapidly and very extensively, if the hydrogen-ion concentration is relatively high, and then gradually decreases and that the extent of the change in this ratio varies directly with the hydrogen-ion concentration.

The Action of the Contractile Vacuole

The results presented by Chalkley (1929) and those presented in the preceding pages show that the volume of *Amoeba proteus* is correlated with the hydrogen-ion concentration of the surrounding medium. The question now arises as to whether or not this correlation is dependent upon the action of the contractile vacuole. This problem was investigated as follows.

Fifty to one hundred amoebae were transferred successively through three beakers each containing 50 cc. redistilled water and left in the last for one hour. During this time many of the amoebae became radiate in form. About 25 of these were selected and put into 50 cc. 0.002 M Ringer solution (pH 6.8) and left 12–15 hours, then an actively moving specimen was selected and measured in the volumescope as previously described (Mast and Fowler, 1935). It was then transferred to test solution, in the depression on a Pyrex glass slide, and covered with a cover-glass, after which the diameter of the vacuole, immediately preceding contraction, and the interval between successive contractions were measured with a Filar micrometer ocular and with a stop watch respectively. This was continued as long as desired, after which the whole process was repeated with other individuals in this and in other solutions. Then the average volume of fluid eliminated by the contractile vacuole per amoeba per minute and the average volume eliminated per minute in percentage of the volume of the amoebae were calculated for each solution used.

There was considerable variation in given amoebae during the period of observation in the different solutions used and in the intervals between successive contractions, but these variations were not specifically correlated with time in any of the solutions except the Ringer-lactose solution, a solution in which the osmotic concentration was relatively very high. In this solution the size of the vacuole decreased and the interval between contractions increased with time and there usually were not more than five contractions before it ceased to function altogether. These statements are substantiated by the following typical results.

In one of the amoebae transferred to Na buffer solution (pH 6.5), the diameter of the first vacuole in the series of ten measured was 2.36 μ, that of the last 2.30 μ, that of the smallest 2.07 μ and that of the largest 2.66 μ.

In one of the amoebae transferred to the Ringer-lactose solution, the diameter of the first vacuole was 2.42 μ and those of the following three were 2.79 μ, 2.69 μ and 2.38 μ respectively, and the intervals between the successive contractions in the series were 2′ 45″, 3′ 5″, 5′ 0″ and 6′ 30″. After this series of contractions was complete the vacuole was continuously observed for 45 minutes. It did not contract during this time but it became smaller, the diameter at the end of three successive 15-minute intervals having been 1.75 μ, 1.66 μ and 1.57 μ respectively. In this amoeba the vacuole ceased contracting 17 minutes and 20 seconds after transfer to the Ringer-lactose solution. The average time required for cessation of contraction in this solution was 18.5 minutes.

The averages of the results obtained directly by observation of amoebae in the different solutions used and those obtained by calculations are presented in Table I.

This table shows that the size of the contractile vacuoles in the amoebae in all the different solutions used except the Ringer-lactose solution and the Na buffer (pH 8) was essentially the same.

In the Ringer-lactose solution the vacuole decreased markedly with time, as stated above, hence the low average diameter of 2.09 μ. The high average diameter of 2.76 μ in the Na buffer (pH 8) appears to have been directly correlated with the size of the amoebae in this solution.

TABLE I

The volume of fluid eliminated by the contractile vacuole in Amoeba proteus *in various solutions.* Temperature, 25° C. Ringer and Na buffer solutions are described in the text. The volume of only three of the nine amoebae in pure water was measured. The average volume for these is 4980 cμ and the average elimination 0.30 per cent of this volume.

Solutions used	Amoebae studied		Contractile vacuoles				
	Number	Av. vol. in 1000 cμ	Number measured	Av. diam. in μ	Av. interval between contractions	Av. vol. of fluid eliminated in cμ per min. per amoeba	Av. percentage of vol. of amoebae eliminated per min.
Ringer 0.002 M pH 6.8	10	1785	100	2.46	3′ 49″	5670	0.31
Ringer 0.002 M pH 6.8 −0.2 M lactose	10	?	42	2.09	9′ 47″	1500	0.008
Na buffer 0.002 M pH 5	5	1753	50	2.29	4′ 5″	4260	0.24
Na buffer 0.002 M pH 6	6	1891	60	2.25	4′ 10″	4620	0.24
Na buffer 0.002 M pH 6.5	6	1619	60	2.22	4′ 4″	3900	0.24
Na buffer 0.002 M pH 7	5	2184	50	2.43	5′ 31″	7380	0.17
Na buffer 0.002 M pH 8	10	3141	100	2.76	4′ 2″	7620	0.24
Pure water	9	1625	90	2.61	3′ 36″	4980	0.30

The table shows that the average interval between successive contractions was relatively small in pure water and Ringer solution and considerably higher but essentially the same in all the Na buffer solutions except pH 7 in which it was relatively very high. It shows that the rate of elimination per unit volume of protoplasm was relatively high in pure water and Ringer solution and considerably lower but essentially the same in all the Na buffer solutions except pH 7, in which it was much lower.

The results obtained consequently indicate that the rate of elimination of fluid by the contractile vacuole in *Amoeba proteus* is practically independent of the hydrogen-ion concentration except in the region of neutrality where it decreases markedly and that it gradually decreases to zero in hypertonic solutions.

Discussion

The results presented in Table I show that in amoebae which have been transferred from Ringer solution to sodium solution of various hydrogen-ion concentrations, there was a change in the rate of elimination of fluid by the contractile vacuole of only 0.07 per cent of the volume of the amoebae per minute. It is consequently obvious that the action of the contractile vacuole was only slightly involved in the changes in the volume of the amoebae observed, in relation to changes in hydrogen-ion concentration (Fig. 1), and that these changes were consequently largely due to the effect of the hydrogen-ion concentration of the solutions used, on the permeability of the surface layer to water.

Table I and other evidence presented above show that in the amoebae which had been transferred from 0.002 M Ringer solution to 0.002 M Ringer solution plus 0.2 M lactose, the rate of elimination of fluid by the contractile vacuole decreased from 0.3 per cent of the volume of the amoebae per minute to zero in an average of 18.5 minutes. No observations were made on the action of the vacuole in amoebae which had been transferred in the opposite direction, but it is highly probable that after such a transfer the vacuole becomes active as rapidly and to the same extent as it becomes inactive after the reverse transfer. If this is true, decrease in volume of amoebae in hypertonic solution is considerably augmented and increase in volume of amoebae in hypotonic solution is considerably retarded, owing to elimination of fluid by the vacuole; that is, the amount of fluid which leaves the amoebae directly through the surface in the hypertonic solution is equal to the increase in volume minus the amount eliminated by the vacuoles and the amount which enters the amoebae directly through the surface in the hypotonic solutions is equal to the increase in the volume of the amoebae plus the amount eliminated by the vacuoles.

Mast and Fowler (1935) calculated the permeability constant for water from results obtained in observations on the increase in the volume of amoebae in hypotonic solutions, but they did not consider the effect of the action of the contractile vacuole on the volume. As stated above, this probably amounted to 0.3 per cent of the volume of the amoebae per minute soon after the transfer to these solutions. The calculated value obtained by them (0.026) is therefore somewhat too small.

In the preceding paper it was demonstrated that after amoebae have been transferred from Ringer solution to this solution plus 0.2 M lactose, they decrease about 15 per cent in volume in 15 minutes and it was demonstrated above that under these conditions they continue to contract for about 18.5 minutes. It is therefore obvious that elimination of fluid through the vacuole continues after considerable fluid has passed out of the body by diffusion and that the action of the vacuole is not immediately dependent upon entrance of fluid and turgidity of the cell. This also obtains for other protozoa (Kitching, 1938, p. 148).

Pitts and Mast (1934), in observations on the gel/sol ratio in *Amoeba proteus*, obtained results which in general support the conclusion reached above, namely that the gel/sol ratio is relatively low in the region of neutrality. They also found that the rate of locomotion is low in this region and Table I above shows that the rate of elimination of fluid by the contractile vacuole is also low in this region. This indicates that the rate of locomotion and the action of the vacuole vary inversely with the fluidity of the cytoplasm and that it is maximum at neutrality. This is probably in some way correlated with the isoelectric point of a prominent protein in the cytoplasm.

The extraordinary changes observed in the gel/sol ratio in calcium solutions indicate remarkably rapid and extensive adjustment but concerning the processes in this adjustment there is no evidence.

Summary

1. As the hydrogen-ion concentration decreases from pH 5.5 the volume of *Amoeba proteus* in solutions containing sodium as the only metallic ion increases to a maximum at pH 6.0, then decreases to a minimum at pH 7.0, and then increases to a second maximum at pH 7.5; and the gel/sol ratio increases to a maximum at pH 6.5, then decreases very extensively to a minimum at pH 7.0, and then increases equally extensively to a second maximum at pH 7.5.

2. In solutions containing potassium as the only metallic ion the volume and the gel/sol ratio decrease to a minimum at pH 6.0 and then increase.

3. In solutions containing calcium as the only metallic ion the volume remains nearly constant, but the gel/sol ratio increases very rapidly and extensively and then gradually decreases; but the extent of change in this ratio varies directly with the hydrogen-ion concentration.

4. The rate of elimination of fluid by the contractile vacuole is practically independent of the hydrogen-ion concentration except in the region of neutrality where it decreases markedly. In hypertonic solutions it gradually decreases to zero.

5. The change in the rate of elimination of fluid by the vacuole in relation to hydrogen-ion concentration is so low in comparison with the change in rate of passage of fluid directly through the surface that it is negligible. The changes observed in the volume of the amoebae in relation to the hydrogen-ion concentration were therefore almost entirely due to changes in the rate of transfer of fluid directly through the surface, i.e. to changes in the permeability of the surface to water.

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